131 Received: May 30, 2012 Accepted: November 11, 2012

INFLUENCE OF HORMONE-VITAMIN-MINERAL TREATMENT OF WÜRTTEMBERG EWES ON REPRODUCTIVE EFFICIENCY IN DEEP OFF-SEASON

Ratko Mijatović¹, Stoja Jotanović^{2*}, Marinko Vekić², Đorđe Savić², Mustafa Podžo³, Blagoje Stančić⁴

 ¹Veterinary Ambulance, Knjaza Miloša 23, Banja Luka 78000, Bosnia and Herzegovina
²Faculty of Agriculture, University of Banja Luka, Bulevar Petra Bojovića 1a, Banja Luka 78000, Bosnia and Herzegovina
³Veterinary Faculty, University of Sarajevo, Zmaja od Bosne 90, Sarajevo 71000, Bosnia and Herzegovina
⁴Faculty of Agriculture, University of Novi Sad, Trg Dositeja Obradovića 8, Novi Sad 21000, Serbia stojaj@yahoo.com

The aim of the study was to investigate the influence of hormone-vitamin-mineral treatment on the reproductive efficiency of Württemberg ewes in deep off-season. The experimental group and the control one consisted of 35 ewes each. Housing conditions, diet, hormonal treatment and insemination were identical for both groups. The difference between the experimental group and the control group was in the use of vitamin-mineral treatment. Ewes were treated with progestagens (30 mg FGA), using intravaginal sponges for 12 days. On the day of sponge removal, all ewes were treated with 700 IU eCG. On the day of the first insemination ewes were treated with 250 IU hCG. Insemination was performed twice with freshly diluted sperm. The experimental group had fertility of 94.29%, fecundity of 137.14% and prolificacy of 145.45%. In the control group these values were 70.59%, 105.78% and 150.00%, respectively. Using the described hormonal treatment, estrus can be successfully induced and synchronized in deep off-season. By applying additional vitamin-mineral treatment reproductive efficiency can be improved, compared to the hormonal treatment only.

Key words: ewe; estrus; hormone; vitamin-mineral treatment; reproductive efficiency

ВЛИЈАНИЕ НА ХОРМОНСКО-ВИТАМИНСКО-МИНЕРАЛЕН ТРЕТМАН ВРЗ РЕПРОДУКТИВНАТА ЕФИКАСНОСТ КАЈ ОВЦИТЕ ОД РАСАТА WÜRTTEMBERG ДЛАБОКО НАДВОР ОД СЕЗОНАТА

Целта на студијата е да се испита влијанието на хормонско-витаминско-минерален третман врз репродуктивната ефикасност кај овците од расата Württemberg длабоко надвор од сезоната. Двете групи (експериментална и контролна) се состоеја од по 35 овци. Условите за сместување, исхрана, хормонален третман и оплодувањето беа идентични за двете групи. Разликата помеѓу експерименталната и контролната група беше во употреба на витаминско-минералниот третман. Овците беа третирани со прогестагени (30 mg FGA), со користење на интравагинални сунѓери, во текот на 12 дена. На денот на отстранувањето на сунѓерот сите овци беа третирани со 700 IU eCG. На денот на првото осеменување овците беа третирани со 250 IU hCG. Осеменувањето беше извршено два пати, со свежо разредена сперма. Кај експерименталната група концепцијата изнесуваше 94,29%, плодноста 137,14% и оплодноста (пролификација) 145,45%. Кај контролната група овие вредности изнесуваа 70,59%, 105,78% и 150,00%, соодветно. Со користење на опишаниот хормонален третман, еструсот може да биде успешно предизвикан и синхронизиран длабоко надвор од сезоната. Со примена на дополнителен витаминско-минерален третман може да се подобри репродуктивната ефикасност во споредба со користење само на хормонален третман.

Клучни зборови: овца; еструс; хормон; витаминско-минерален третман; репродуктивна ефикасност

INTRODUCTION

The hormonal method is one of the most efficient methods for managing sheep reproduction. By using it, induction and synchronization of estrus outside the breeding season is achieved, it reduces the debt of the anestric period, increases the number of lambs obtained per ewe per year, allows to get three lambings in two years, with uniformity of weight and age of lambs, and planning production when the market price is the best (Vilić, 2007).

Estrus induction in off-season is usually done by the Chrono-gest or the Veramix method, using intravaginal sponges in combination with application of eCG on the day of sponge removal. According to these methods, ewes should be treated with eCG immediately before or inside six hours from the time of sponge removal. Greyling et al. (1997) suggest that the degree of induction of estrus in ewes treated with a combination of progestins and eCG is 85-90%. According to Cognie et al. (1992), without application of eCG, ovulation occurs later, and it is difficult to determine the optimal moment for insemination. The eCG dose required for successful induction of estrus depends on breed characteristics, general health condition, interval since last lambing and parity of lambing. Popovski (2001) suggested doses of eCG of 500 IU for the sheep in the physiological anestrus (January - July period) and 300-400 IU in the usual breeding season. The height of the eCG dose significantly affects the average number of lambs born in the induced estrus. Covan et al. (2003) reported that hCG has a similar function as the LH and accelerates ovulation. Research carried out by Simonetti et al. (2000) and Viñoles et al. (2001), showed that sheep came into estrus during the period of 24-144 hours after the sponge removal, with the highest incidence between 30 and 60 hours. Accorindg to Podžo et al. (2003), the most significant factors for success of the hormonal method are the phase of the reproductive cycle in which the treatment is done, the type and the dose of the used hormonal preparations, the breed and the age of sheep, the nutrition, the general health state, the moment of insemination, and the quality of stud rams or semen for artificial insemination.

The aim of this study was to determine if estrus can be induced in deep off-season with the described hormonal treatment, as well as the impact of additional vitamine-mineral treatment on achieved parameters of reproductive efficiency.

MATERIAL AND METHODS

The study was conducted on a Württemberg sheep farm, during the period June 2009 – January 2010. The total of 70 sheep were divided into two groups of 35 individuals. All sheep were in good health state, with an average weight about 70 kg. Animals were in a parity range from one to four lambings. Sheep were kept outdoors. The diet was based on pasture with small amounts of concentrated feed. Lambs were weaned 30 days before the beginning of the experiment, and sheep were intensively fed with concentrated feed during this period, in order to acheive a "flushing" effect.

As preparation intravaginal progestagen sponges were used. Intravaginal sponges containing 30 mg fluorogestan acetate (Chrono-gest, Intervet-Boxmer, The Netherlands) were placed with an original plastic applicator. The treatment lasted for 12 days, with the day of application as day 0. On the day of sponge removal intravaginaly all sheep were treated with 700 IU eCG (Folligon, Intervet-Boxmer, Netherlands), and 48 hours later, with 250 IU hCG (Pregnyl, NS Organon, Oss., Netherlands), in order to get synchronized ovulation. The experimental group of sheep was additionally treated with 5 ml of vitamin complex (Vitamin AD3E, Pliva, Zagreb, Croatia) and 10 ml mineral solution (Calfoset, Krka, Novo Mesto, Slovenia), on the day of sponge application.

Sheep were inseminated twice, with fresh diluted semen prepared two hours before use. The first insemination was performed 48 hours after the treatment with eCG, the second 24 hours later (day 15. and 16. from the sponge removal). The volume of the single insemination dose was 0.3 ml, with about 400 million progressively motile spermatozoa.

During the research data on values of fertility, fecundity, prolificacy, litter size and vitality of the lambs were monitored. As methods of statistical analysis we used the descriptive analysis and comparison by t-test.

RESULTS

Achieved parmeters of reproductive efficiency are shown in Table 1.

Table 1

Parameters of reproductive efficiency of examined sheep (%)

Parameter	Gro	Total		
Farameter	Experimental	Control		
Fertility	94,29±3,92 ^a	70,59±7,81 ^b	82,61±4,56	
Fecundity	137,14±6,41 ^a	105,88±3,81 ^b	121,74±4,17	
Prolificacy	145,45±6,69 ^a	$150,00{\pm}7,85^{a}$	147,37±5,09	

 a,b Values in the same row marked with different letters in superscript are statistically significantly different (p<0.01)

A highly statistically significant difference (p<0.01) was found in values of fertility and fecundity, while in values of prolificacy such a difference did not exist. The control group had a slightly higher value of prolificacy, but the number of lambed sheep and the total number of lambs were smaller in absolute terms.

The litter size and the viability of lambs in the first 24 hours of life are shown in Table 2.

Table 2

Group	Individuals		Twins		Triplets		Quadruplets	
	%	Viability (%)	%	Viability (%)	%	Viability (%)	%	viability (%)
Experimental	63,67±8,37 ^a	100	30,30±8,00 ^a	90	3,00±2,98	100	3,00±2,98 ^a	0
Control	58,30±10,09 ^a	85,71	37,50±9,88 ^a	94,44	-	-	4,20±4,07 ^a	75

Litter size and viability of lambs in the first 24 hours of life

^{a,b} Values in the same row marked with different letters in superscript are statistically significantly different (p<0.05).

No statistically significant difference (p<0.05) was found between the experimental group and the control group in relation to the litter size. The control group had more twins and quadruplets than the experimental one, and their viability was greater than in the experimental one. Incidence of quadruplets is not characteristic for this sheep breed, and can be interpretated as accidental, rather than as a result of the used treatment.

DISCUSSION

Induction and synchronization of estrus in off-season in our study was based on extending the luteal phase of the cycle by applying a combination of progestogens and gonadotropins. In addition to the hormonal treatment, the experimental group of sheep was treated with vitamin-mineral preparations, to examine their impact on the improvement of reproductive parameters. For a better interpretation of shown results, it is important to note that the experiment was conducted in deep off-season, which certainly had an impact on the reproductive indicators.

The achieved fertility of the experimental group and the total fertility rate is better than the results of Simonetti et al. (2002), who achieved fertility of 54.32% and 76.47% after using MAP and two different doses of eCG (375 IU and 400 IU) and dual insemination of merino sheep with fresh diluted semen. These authors found no statistically significant difference in fertility in relation to the number of inseminations, but with dual in-

semination they got 6-10% more lambs in absolute values. Crosby et al. (2003) achieved fertility of 72% by applying the treatment of progesterone and eCG. Higher values of fertility in comparison to our Zeleke et al. (2005) realized, who achieved fertility of 85.30% and 97% in Dorper sheep by using MAP and FGA, respectively. Fertility of the experimental group is better than the results of Šahinović et al. (2006). These authors achieved a fertility rate of 83.35% on Württemberg sheep in late anestrus by using natural mating, while the value of fertility in the usual mating season was 94.4%. Results achieved in our study are consistent with Vilić (2007), who achieved fertility of 88.00% after the hormonal treatment of Württemberg sheep with combination of FGA and eCG in the late anestrus season. Similar results of fertility in induced estrus were achieved by other authors (Redmer et al. 2000; Safdarian et al. 2006; Dogan and Nur, 2006; Krid et al. 2006; Todini et al., 2007).

Fecundity of the experimental group is larger than the results of Zeleke et al. (2005), who achieved fecundity of 120.8% and 131.1% after the treatment of the Dorper sheep with MAP and FGA, respectively, and Safdarian et al. (2006) who realized fecundity of 133% after the treatment of the Karakul sheep with FGA and eCG. Both groups had a higher fecundity compared to the results of Kridli et al. (2006), who had fecundity of 77.70% in the Awassi sheep using FGA and eCG.

According to Stančić and Veselinović (2002) prolificacy depends on the genetic and paragenetic factor, but primarily on genetically determined

ovulation value for a particular breed. Petrović (1992) had the minimal value of prolificacy for the Württemberg sheep (120.05%), and the highest (162.00%) was found by Valter (1989). Skalicki et al. (1998) achieved prolificacy of 144.00% in the trial conducted on the Württemberg sheep. Prolificacy of the experimental group was slightly lower compared to the control one, suggesting that the used vitamin-mineral treatment had no significant influence on the litter size. These values and approximately equal number of lambs obtained per lambed ewe in both groups confirm the assertion that the litter size depends primarily on the breed, and to a lesser extent to paragenetic factors. The obtained values of prolificacy are lower compared to the results of Crosby et al. (2003), Todini et al. (2007) and Vilić et al. (2007), who achieved values of 174%, 220% and 161%, respectively. Our results of prolificacy are better compared to the ones of Hussein et al. (2004), Kridli et al. (2006), Safdarian et al. (2006) and Sahinović et al. (2006). This difference can be attributed to the season in which estrus was induced, and certainly to the used breed of sheep.

The shown litter size and the lack of statistical significance of differences can be attributed to the breed characteristics of the used sheep, which is selected on meat and wool production, and have mostly one, rarely two lambs in litter (Mitić, 1987). Incidence of triplets and quadruplets is not characteristic for this sheep breed, and can be interpretated as accidental, rather than as a result of the used treatment. This is supported by the data on the viability of lambs in the first 24 hours of life, which showed that there was no mortality in triplets, whereas mortality was very high in quadruplets (87.5%). No statistically significant difference was found between experimental and control groups in the total viability of obtained lambs, although the percentage of viability was slightly higher in the control group. However, the absolute number of lambs in the experimental group was higher, so it can be said that their viability was higher than in the control group.

CONCLUSION

The conclusion of our study is that induction and synchronization of estrus in deep off-season can successfully be done with the use of the described hormonal treatment. For achieving this, it is necessary to prepare dams by flushing nutrition. Additional treatment with vitamins and minerals had a positive influence on the reproductive efficiency in general, but prolificacy remained in limits for a used breed.

REFERENCES

- Cognie, Y. (1992): Progress in reproduction techniques in sheep. *Proceedings of the World sheep and wool con*gress, Buenos Aires, 111–129.
- [2] Çoyan, K., Ataman, M. B., Erdem, H., Kaya, A., Kasikci, G. (2003): Synchronization of estrus in cows using double PGF₂ alpha, GnRH–PGF₂ alpha and hCG-PGF₂ alpha combination. *Revue Méd. Vét.*, 154, 91–96.
- [3] Crosby, T. F., Boland, M. P., Gordon, I. (1991): Effect of progestagen treatments on the incidence of oestrus and pregnancy rates in ewes. *Anim. Reprod. Sci.*, 24, 109– 118.
- [4] Dogan, I., Nur, Z. (2006): Different estrous induction methods during the non-breeding season in Kivircik ewes. *Veterinarnia medicina*, 51, 133–138.
- [5] Greyling, J. P. C. (1997): The effect of dose of prostaglandin in the synchronization of oestrus in the Boer goat doe. *Proceedings of the Sixth International Conference in Goats*, Beijing, 826.
- [6] Husein, M. Q., Ababneh, M. M., Abu-Ruman, D. S. (2007): The effects of short or long term FGA treatment with or without eCG on reproductive performance of ewes bred out-of-season. *Am. J. Anim. Vet. Sci.*, 2, 23–28.
- [7] Kridli, R. T., Husein, M. Q., Muhdi, H. A., Al-Khazaleh, J. M. (2006): Reproductive performance of hormonallytreated anestrous Awassi ewes. *Anim. Reprod.*, 3, 347– 352.
- [8] Mitić, N. (1987): Sheep breeding. Institute of Textbooks and Teaching Aids, Belgrade.
- [9] Petrović, P. M. (1992): Examination of reproductive and productive performances of Württemberg sheep (Merinolandschaf). PhD thesis, University of Belgrade (in Serbian).
- [10] Popovski, K. (2001): Use of endocrinology in reproduction. University of Skoplje, Skoplje (in Macedonian).
- [11] Redmer, D. A., Haugen, R. G., Stenbak, T. K., Arnold, D. R., Toutges, M. J., Berginski, H. R., Navanukra, W. C., Krisch, J. D., Reynolds, L. P. (2000): Effects of gonad-otropin on induced of oestrus and pregnancy rate in ewes synchtonized with Syncho-Mate-B (SMB) and subjected to laparoscopic artificial insemination (LAI) during the breeding season. *Western Dacota Sheep Day*, Report No 41, 93–96.
- [12] Safdarian, M., Kafi, M., Hashemi, M. (2006): Reproductive performance of Karakul ewes following different oestrous synchronization treatments outside the natural breeding season. S. Afr. J. Anim. Sci., 36, 229–234.
- [13] Simonetti, L., Blanco, M. R., Gardon, J. C. (2000): Estrus synchronization in ewes treated with sponges impregnated with different doses of medroxyprogesterone acetate. *Small Ruminant Res.*, 38, 243–247.

- [14] Simonetti, L., Ramos, G., Gardón, J. C. (2002): Effect of estrus synchronization and artificial insemination on reproductive performance of merino sheep. *Braz. J. Vet. Res. Anim. Sci.*, 29. 143–146.
- [15] Skalicki, Z., Ristevski, T. (1998): Productive performances of Württemberg sheep breed. *Contemporary Agriculture* 48, 91–96 (in Serbian).
- [16] Stančić, B., Veselinović, S. (2002): Biotechnology in Rproduction of Dmestic Aimals. Faculty of Agriculture, Novi Sad (in Serbian).
- [17] Šahinović, R., Vilić, H., Mujić, E., Bašić, N., Alibabić, V. (2006): Productive and reproductive performances of Württemberg sheep breed in Unsko-Sanski region and results of hormonal treatment, *Contemporary Agriculture* 55, 177–181 (in Serbian).
- [18] Todini, L., Malfatti, A., Barbato, O., Costarelli, S., Debenedetti, A. (2007): Progesterone plus PMSG priming in

seasonally anovulatory lactating Sarda ewes exposed to the ram effect. J. Reprod. Dev., 53, 437–441.

- [19] Vilić, H. (2007): Hormonal method as method for improving reproductive efficiency of sheep in Unsko-sanski region. MSc thesis, University of Novi Sad (in Serbian).
- [20] Viñoles, C., Forsberg, M., Banchero, G., Rubianes, E. (2001): Effect of long-term and short-term progestagen treatment on follicular development and pregnancy rate in cyclic ewes. *Theriogenology*, 55, 993–1004.
- [21] Walter, R. (1989): *Das Merindandschaf*. Der Bayerische Schafhalter, 4, 106–109 (in German).
- [22] Zeleke, M., Greyling, J. P. C., Schwalbach, L. M. J., Muller, T., Erasmus, J. A. (2005): Effect of progestagen and PMSG on oestrous synchronization and fertility in Dorper ewes during the transition period. *Small Ruminant Res.*, 56, 47–53.